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**13. ABSTRACT (Maximum 200 Words)**

Using a rapid autopsy program at our institution, we have found that prostate cancer not only metastasizes to bone, but also to the liver, dura, retroperitonium, and lung. This proposal focuses on a novel proteolytic mechanism that inactivates the metastasis suppressor protein, E-cadherin in prostate cancer metastasis. Alterations in E-cadherin mediated cell-cell adhesion contribute significantly to defects in cellular attachment found in most human carcinomas, including adenocarcinoma of the prostate gland. We have strong evidence suggesting that E-cadherin is truncated and inactivated by the ADAM15 metalloproteinase during the metastatic progression of prostate cancer. Preliminary results indicated that transfection of ADAM15 into human prostate cancer cells resulted in extensive cleavage of E-cadherin and shedding of the E-cad$^{80}$ fragment into cell culture media. Together, these findings strongly suggest ADAM15 may support growth of metastatic prostate cancer cells by disrupting E-cadherin-mediated adhesion. Despite advances in management of prostate cancer the metastatic progression of this disease remains incurable. It is critical that the molecular mechanisms underlying common metastatic processes are elucidated to allow for development of novel therapeutics to treat metastatic disease. ADAM15 is particularly intriguing as potential therapeutic target, due to extensive research focus on metalloproteinase inhibitors in the pharmaceutical industry.

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## INTRODUCTION

A key determinant in the metastatic progression of prostate cancer is the dissociation of cancer cells from the primary tumor that may result from inadequate cell adhesion. In tumors of epithelial origin, the disruption of cellular adhesion appears to arise, in part, through alterations of the E-cadherin cell-adhesion system. In our original proposal we hypothesized that the disintegrin metalloproteinase, ADAM 15, is closely associated with the metastatic progression of prostate cancer and could possibly cleave E-cadherin into proteolytic fragments. Examination of both cDNA and tumor microarrays demonstrated increased expression of ADAM-15 in metastatic prostate cancer. It was also important to note that the chromosomal location for ADAM-15, on 1q21, is a region of specific high-level amplification in prostate cancer metastasis. Taken together, this information provided a compelling rationale for the proposed studies and supports our central hypothesis: ADAM-15 specifically targets the extracellular domain of E-cadherin and disrupts the adhesive integrity of epithelium during the metastatic progression of prostate cancer. Not only will the proposed studies address the functional role of ADAM-15 in the metastatic transformation of prostate epithelial cells; these results may also justify future studies pursuing ADAM-15 as a direct therapeutic target for metastatic prostate cancer.

## BODY

**Over-expression of ADAM15 in minimally malignant prostate epithelial cells to determine if this over-expression cleaves E-cadherin and induces a malignant phenotype.** The intent of aim 1 was to achieve stable high-level expression of ADAM15 in LNCaP cell line and determine if ADAM15 elevation induces E-cadherin cleavage as well as a malignant phenotype in this minimally malignant prostate cancer cell line. ADAM15 was tagged with GFP on its C-terminus and transfected into LNCaP cells. ADAM15-GFP over-expressing LNCaP cells were verified via western blotting and immunohistochemistry by our laboratory (Figure 1). The inactive precursor form of ADAM15 is a 110 kDa protein which is converted into the 90 kDa active form by the pro-protein convertase furin. We will use the stable LNCaP cell lines (LNCaP ADAM15-GFP) to perform cell motility, invasion and anchorage-independence assays to determine malignant potential of theses cell lines.

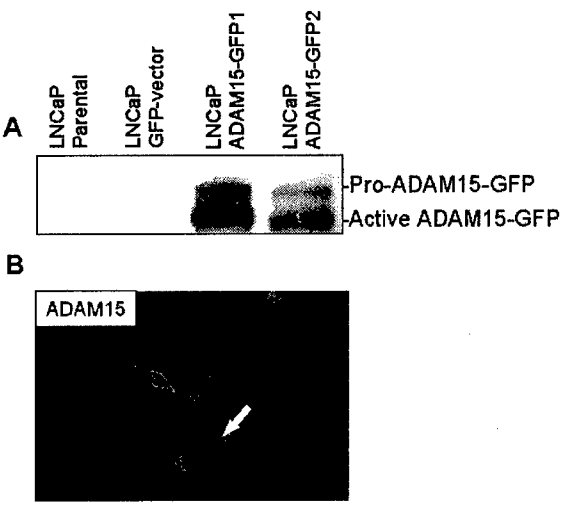


**Figure 1. ADAM15-GFP LNCaP Cells.** LNCaP cells were transfected with vector-GFP or ADAM15-GFP vector constructs. **(A)** Western blot showing the exogenous ADAM15-GFP in ADAM15-GFP transfected LNCaP cells. **(B)** Immunocytochemistry showing membranous staining (arrow) of ADAM15 in LNCaP cells.

**To determine if ADAM15 knockdown reduces the cleavage of E-cadherin as well as the metastatic phenotype of highly aggressive prostate cancer cells.** The results from aim 1 may indicate the ADAM15 promotes a malignant phenotype; however this does not confirm that ADAM15 is directly inducing this phenotype. Thus, the intent of this aim is to confirm that ADAM15 is specifically inducing the malignant phenotype seen by using reverse genetics. To accomplish this task, we will utilize small interfering (si)-RNA-mediated knockdown of ADAM15 using a short hairpin (sh)-RNA construct. We have successfully reduced ADAM15 expression in PC3 cells using transient transfections with two separate ADAM15 siRNA oligos (siRNA1 and siRNA3) (Figure 2). Next we aim to produce stable ADAM15 siRNA knockdown in the highly malignant PC-3 cell line using a lentiviral system. Once expression has been verified, we will use the stable ADAM15 siRNA treated PC-3 cell lines to perform cell motility, invasion and anchorage-independence assays as mentioned previously to determine metastatic potential of these cell lines. PC-3 cells are motile, invasive, and anchorage independent. We anticipate that ADAM15 siRNA expressing PC-3 cells will exhibit less motility, invasiveness and anchorage independence in comparison to vector control.

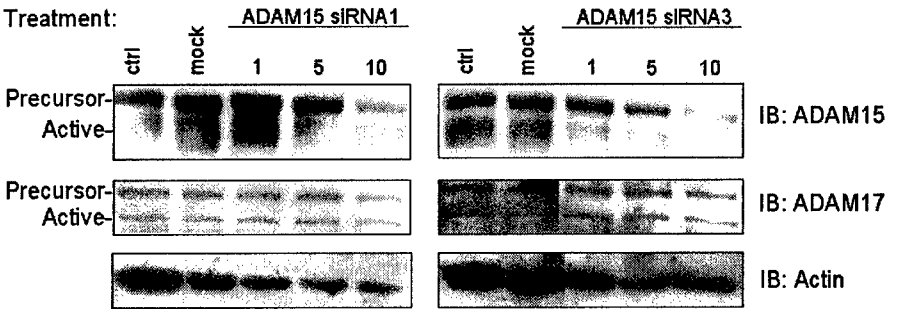


**Figure 2. Transient ADAM15 siRNA Treatment.** PC-3 cells were treated with control GFP vector (mock), 1µg, 5 µg, and 10 µg of 2 different ADAM15 siRNA oligonucleotides or vehicle (ctrl). Cells were harvested 48 hrs. post treatment and Western blotting was performed. Immunoblotting (IB) was performed for ADAM15, ADAM17 (as control) and actin.

## ACCOMPLISHMENTS:

1. ADAM15-GFP constructs were completed and successfully expressed in LNCAP cells.
2. Small interfering (si)-RNA-mediated knockdown of ADAM15 has been achieved in transient models. We are close to completing stable expression using viral vectors.
3. We have started the first round of intratibal injections using our ADAM15-GFP transfectants.

## REPORTABLE OUTCOMES:

We have created several cell lines that express ADAM15-GFP. Abdo Najy who is a graduate student in my lab has applied for and NCI predoctoral fellowship as well as this years DOD predoctoral fellowship. Lastly we are very close to submitting a manuscript focusing on the examination of ADAM15 expression not only in prostate cancer but in breast cancer as well. While this study was not a direct aim of the funded proposal this report will have bearing on the research as a whole. Much of the data in this paper was provided as preliminary data in the funded proposal.

## CONCLUSIONS:

In summary this study to date has yielded two important categories of information:

1. Necessary reagents for the remainder of the study are being generated and versified.
2. The clinical data to be published examining the expression of ADAM15 in breast and prostate cancer will be a solid foundation of clinical relevance with which to build the functional story on.